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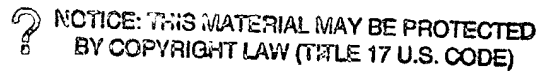
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THE BIOLOGY OF THE EOSINOPHILIC LEUKOCYTE

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ABSTRACT

The eosinophil is characterized by specific cytoplasmic granules that contain a series of cationic toxins able to kill many targets, including helminths, protozoa, bacteria, and other cells. In bronchial asthma, considerable evidence exists that the eosinophil releases granule proteins, especially the major basic protein (MBP), which in turn mediate tissue abnormalities. Among eosinophil-activating factors, IL-5 has been associated with helminth infection and hypersensitivity diseases and would appear to be an attractive target for pharmacological intervention.

INTRODUCTION

Information about the eosinophil has rapidly expanded over the past several years. The discovery that the eosinophil is a likely mediator of bronchial asthma (1) has increased interest regarding the eosinophil in asthma and other respiratory diseases. The occurrence of the eosinophilia-myalgia syndrome (EMS) as an epidemic related to the ingestion of tainted L-tryptophan has further stimulated awareness of the eosinophil (2). This heightened interest is manifested by a dramatic increase in publications dealing with the eosinophil: for example, before 1975, approximately 100 publications per year could be identified from the National Library of Medicine data base under the search term eosinophil; in 1990 over 900 articles on eosinophils were published. In this review, we briefly present

information on the biology of eosinophil function and relate this information to ongoing investigations of the role of eosinophils in parasitism and in a hypersensitivity disease, namely bronchial asthma.

EOSINOPHILS AND THEIR CONSTITUENTS

Morphology

As reviewed in detail elsewhere (3), eosinophils contain three types of granules: primary granules, which are round, uniformly electron dense, and characteristically present in eosinophilic promyelocytes; specific or secondary granules, which are composed of an electron-dense core and an electron-lucent matrix; and small granules, of which relatively little is known except that they contain acid phosphatase and arylsulfatase. Eosinophils also contain lipid bodies, nonmembrane-bound lipid-rich inclusions that are present in many types of cells and that incorporate ^3H -arachidonate (4). Although normal eosinophils are usually circular or ovoid when observed by light microscopy, cells with one or more pseudopods have been seen in human blood, sputum, bone marrow, and nasal smears (5); they are termed medusa cells to highlight the presence of pseudopods. Although the significance of medusa cells is unknown, they may be related to alterations of the cell during the course of its death.

Cellular Constituents

The constituents of the eosinophil can be divided into molecules associated with membranes and molecules associated with granules.

MEMBRANE PROTEINS Receptors for immunoglobulins and complement, including IgG, C1q, C3b/C4b (CR1), iC3b (CR3), and C5a, are present on eosinophils (reviewed in 3 and 6). The IgG receptor is the low-affinity Fc- γ R II (CD32) (7). The absence of Fc- γ R III on eosinophils and its presence on neutrophils has proven to be a useful means for eosinophil purification by negative selection (8). The IgE receptor is of low affinity and appears to be similar, if not identical, to CD23 present on B cells and monocytes.

When eosinophils are incubated with IgG-coated schistosomula of *Schistosoma mansoni*; they degranulate and kill the parasite (9). To mimic these conditions, beads coated with immunoglobulins, including IgG, IgA, and, most potently, secretory IgA (sIgA), have been used to stimulate eosinophil degranulation (10). Eosinophil degranulation is not inhibited by glucocorticoids (11), but it is inhibited by agents increasing intracellular concentrations of cyclic AMP (cAMP), such as phosphodiesterase inhibitors (12), and by pertussis toxin (at least two pertussis-sensitive G proteins

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appear to be involved) (13). The information that sIgA potentially stimulates eosinophil degranulation is interesting in light of the localization of eosinophils in tissues associated with epithelial surfaces, including the gastrointestinal tract, the respiratory tract, and the skin. Comparison of eosinophil degranulation stimuli revealed that sIgA-coated Sepharose beads were the most potent, followed by the calcium ionophore, A23187, formyl-methionyl-leucyl-phenylalanine (FMLP) and more distantly by IgG-coated beads and phorbol myristate acetate (PMA) (13).

Eosinophils possess receptors for several cytokines, including interleukin (IL)-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (14, 15), and presumably for interferon (IFN)- γ , IFN- β , IFN- α , and tumor necrosis factor (TNF- α) (16). Eosinophils also have receptors for platelet-activating factor (PAF) (17) and presumably for leukotriene (LT) B_4 in that this is a potent chemotactic agent for eosinophils (18). Further evidence exists that eosinophils also have receptors for estrogens, for glucocorticoids, and for β -adrenergic agonists.

Adhesion molecules, including CD11a, Mac I (CD11b), P150-95- α (CD11c), and the common beta chain (CD18) receptors, have been identified on eosinophils (7). The possession of these β 2 integrins permits eosinophils to adhere to intercellular adhesion molecule-1 (ICAM-1) and CR3 (19). However, neutrophils also would be able to adhere to endothelial cells via these same mechanisms. Recently, specific eosinophil adherence to IL-1-stimulated human umbilical cells has been shown to be dependent on the very late activation antigen-4 (VLA-4) integrin receptor and on endothelial cell expression of the vascular cell adhesion molecule-1 (VCAM-1) (20). Thus, these results suggest that eosinophils employ VCAM-1 for specific adherence. Interestingly, the occurrence and function of VCAM on endothelial cells is increased by IL-4 (21), a cytokine associated with TH-2.

Eosinophils also express membrane CD4, usually associated with T helper cells (22); the eosinophil CD4 can bind glycoprotein 120 of the human immunodeficiency virus type I (HIV-1 gp120). Further, three CD4-binding ligands [HIV-1 gp120, bivalent anti-CD4 monoclonal antibody, and lymphocyte chemoattractant factor (23)] stimulate the migration of eosinophils (24). Eosinophils cultured in the presence of murine fibroblasts and GM-CSF synthesize and express HLA-DR protein (25). In antigen-presenting cells, class II protein mediates the interaction with T cells by presentation of processed antigen to CD4+ lymphocytes (26). Preliminary evidence suggests that eosinophils induced to express HLA-DR by in vitro culture with GM-CSF can act as MHC class II restricted antigen-presenting cells (27).

EOSINOPHIL GRANULE PROTEINS The eosinophil contains four pre-

dominant cationic molecules, including eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). The biochemical characteristics of these molecules were recently reviewed in detail (3) and, therefore, the properties of these granule proteins are summarized only briefly here.

Major basic protein (MBP) MBP consists of a single polypeptide chain of 117 amino acids. It is rich in arginine, has a molecular weight of approximately 14,000, and has a calculated isoelectric point of 10.9 (3). Interestingly, the MBP cDNA specifies a prepromolecule with a 90 amino acid pro-sequence followed by the 117 amino acid sequence of MBP. The 90 amino acid pro-portion is enriched in acidic amino acids, especially glutamic acid, and its isoelectric point is 3.9. The combination of pro-portion and MBP yields a molecule of 207 amino acids with approximately equal numbers of strongly basic and acidic amino acids and an isoelectric point of 6.2.

Although several possibilities exist for the functions of the MBP pro-portion, one possibility seems particularly attractive, namely that proMBP protects the cell from the toxic effects of MBP during the transport of proMBP from the Golgi apparatus to the eosinophil granule. MBP is localized in the core of the eosinophil granule, and it is present in basophils, albeit in quantities significantly less than in eosinophils. MBP can also be identified in mast cells, but here it appears that the mast cell endocytoses MBP and that mast cells from normal tissues (not containing eosinophils) are MBP-negative (28). MBP is a potent toxin able to damage various parasites, both helminths and protozoa, to kill bacteria and mammalian cells, to stimulate histamine release from basophils and mast cells, and to activate neutrophils and platelets (3). MBP is able to cause bronchospasm and increased activity to inhaled methacholine when instilled into monkey lung (29); these effects are neutralized by polyglutamic acid (30).

Eosinophil cationic protein (ECP) ECP is a strikingly basic protein, pI 10.8, whose partial end-terminal amino acid sequence has marked homology to that of EDN and to pancreatic ribonuclease. Analysis of the cDNA of ECP reveals that it codes for a preproprotein of 160 amino acids and a protein of 133 amino acids with M_r 15,600. The amino acid sequence shows 66% identity to EDN and 31% to human pancreatic ribonuclease. Thus, ECP is a member of the ribonuclease gene superfamily, the other members of which include EDN, pancreatic ribonuclease, and human angiogenin. The ECP gene is localized to the q24-q31 region of human chromosome 14 (3). ECP is localized to the eosinophil granule matrix; its functions include marked toxicity to bacteria, to helminths and protozoa, and to mammalian cells.

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Eosinophil-derived neurotoxin (EDN) EDN was so named because it is able to cause a neurotoxic reaction when injected into the brains of experimental animals. As noted above (in the section on ECP), EDN is homologous to pancreatic ribonuclease and is thus a member of the ribonuclease superfamily. EDN cDNA has been cloned and it encodes a 134 amino acid polypeptide with M_r of 15,500. The amino acid sequence of EDN is identical to that reported for human urinary ribonuclease and to the amino-terminal sequence of human liver ribonuclease. Like ECP, EDN is localized to chromosome 14, and it is present in the eosinophil granule matrix. EDN causes the Gordon phenomenon when injected into rabbits, and EDN and ECP are essentially equal in potency in this assay (31). Alkylation of EDN with iodoacetic acid destroys its ribonuclease activity and also its neurotoxic activity, which suggests that neurotoxic activity is dependent on ribonuclease activity (32). In contrast to the other eosinophil granule proteins, which are potent toxins, EDN was only weakly toxic to parasites and to mammalian cells (3).

Eosinophil peroxidase (EPO) The partial amino acid sequence of EPO has been determined and its cDNA cloned. The cDNA coded for a 381 base pair pro-sequence, a 333 base pair sequence corresponding to the coding region of the EPO light chain, and a 1392 base pair sequence corresponding to the EPO heavy chain and a 452 base pair untranslated 3' region. The light and heavy EPO subunits correspond to 12,712 and 53,011 M_r proteins with isoelectric points of 10.8 and 10.7, respectively. The sequence of EPO is strikingly similar to that of myeloperoxidase (MPO) and thyroid peroxidase; EPO belongs to a multigene family that also includes a lactoperoxidase (3). EPO is localized to the matrix of the crystalloid-containing granules.

EPO functions both as a cationic toxin in the absence of hydrogen peroxide and as a peroxidase in the presence of hydrogen peroxide. The functions of EPO have been extensively studied on the presumption that it acts as a peroxidase and is able to generate hypohalous acids. These results, utilizing iodide and bromide, showed that EPO was able to kill a variety of targets, including parasites, bacteria, viruses, microplasma, and fungi. Although prior results have shown that EPO prefers bromide over chloride, more recent data indicate that EPO prefers thiocyanate over bromide by at least 100-fold. The product of the interaction of EPO with thiocyanate is hypothiocyanous acid, which is a weak, primarily sulfhydryl-active oxidant. As a cationic toxin in the absence of H_2O_2 and halide, EPO is able to kill parasites and mammalian cells (3).

Charcot-Leyden crystal (CLC) protein (lysophospholipase) Eosinophils produce distinctive hexagonal bipyramidal crystals; the protein comprising

these crystals possesses lysophospholipase activity (3). CLC cDNA has recently been cloned, and the deduced amino acid sequence is strikingly homologous to various lectins (33). Although initial results suggested that CLC was present in the membrane of the eosinophil, more recent data indicate that CLC can be localized to eosinophil granules devoid of crystalline inclusions that seem to be persisting primary granules. The role of CLC in disease is as yet obscure, although recent information suggests that it degrades lysophosphatidylcholine, an important component of pulmonary surfactant (33), and the resulting increase in surface tension might account in part for the tendency of patients with asthma to develop atelectasis.

MEDIATOR PRODUCTION

Eosinophils produce PAF, which can increase cutaneous permeability and smooth muscle contraction and which is a potent stimulator of many cells, including neutrophils, macrophages, and platelets (3). PAF is also a potent chemotactic factor for eosinophils. Eosinophils also preferentially synthesize LTC₄, another potent mediator causing bronchoconstriction and changes in vascular permeability (3), whereas neutrophils predominantly elaborate LTB₄.

EOSINOPHILOPOIESIS

Eosinophils are produced in the bone marrow and then released into the peripheral blood, where they circulate before migrating into the tissues (34). Recent studies show that three eosinophil-active cytokines are able to stimulate colony formation by eosinophil precursors, namely IL-5, IL-3, and GM-CSF; among these, IL-5 appears to be dominant (35). Similarly, human umbilical cord blood cells can be driven to eosinophil differentiation utilizing IL-5 (36). The eosinophilia associated with helminth infection is abolished by pretreating animals with antibodies to IL-5 (37, 38). In humans, serum levels of IL-5 are elevated in patients with onchocerciasis, and the elevations of IL-5 precede the peak of blood eosinophilia; in contrast, IL-3 and GM-CSF levels were not detectable in sera from these patients (39). Furthermore, patients treated with IL-2 developed striking eosinophilia, elevations of blood and urine MBP, and increased serum levels of IL-5 (40). Lastly, IL-5 transgenic mice developed selective eosinophilia (41, 42), and the eosinophilia was blocked by antibody to the IL-5 receptor (43). Because in humans IL-5 appears to have little effect on B-cell function (44), IL-5 may be a particularly appealing target for therapeutic intervention.

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EOSINOPHIL ACTIVATION

At this writing, it appears that the major factors causing eosinophil activation are the same cytokines implicated in eosinophil colony formation, namely IL-5, GM-CSF, and IL-3 (45). It seems likely that these activating factors are produced by mononuclear cells, including T cells and monocytes (3). In the 1970s, studies of a T-cell-derived factor, termed the eosinophil stimulator promoter (ESP), revealed that ESP enhanced the ability of eosinophils to kill targets *in vitro*. Recent studies of ESP show that its activity is attributable to a combination of GM-CSF and IL-5 (46). Additional information supporting the concept of eosinophil activation and identifying a marker for such activation comes from studies of light density eosinophils (3). Whereas the blood of normal individuals contains fewer than 10% eosinophils with density less than 1.082, individuals with eosinophilia may have striking increases in light density eosinophils (47, 48). In addition, the numbers of light density eosinophils directly correlate with the degree of peripheral blood eosinophilia. Light density eosinophils are metabolically more active than their normodense counterparts, show increased oxygen consumption and increased ability to generate superoxide anion, show increased production and/or releasability of LTC₄ after incubation with IgG-coated beads or calcium ionophore, and show potent cytotoxic activity for antibody-coated targets (3). Analyses of the factors responsible for the induction of light density eosinophils, and thus eosinophil activation, have demonstrated that culture of eosinophils with growth factors including GM-CSF, IL-3, IL-5, IFN- α , IFN- γ , alone or in the presence of endothelial cells and/or fibroblasts, generates light density activated eosinophils (45). The enhancement of eosinophil survival is specific for eosinophil-activating factors, e.g. IL-5, but not for granulocyte colony-stimulating factor (G-CSF) (49). Therefore, when normodense eosinophils are activated by growth factors, they become activated light density cells.

The realization that the occurrence of light density eosinophils reflects prior eosinophil activation has stimulated interest in these cells among clinical investigators. Light density eosinophils occur in numerous diseases, including the hypereosinophilic syndrome, parasitic diseases, and bronchial asthma (3). The light density eosinophils from patients with asthma elaborated greater quantities of LTC₄ than did eosinophils of normal density, and bronchial provocation with antigens increased the percentage of light density eosinophils in the peripheral blood, which suggests that eosinophil activation was occurring (50). Furthermore, eosinophils in the hypereosinophilic syndrome are mainly light density eosinophils (48), and IL-5 activity can be demonstrated in the serum of patients with the hypereosinophilic syndrome (51).

In addition to T-cell-derived factors, eosinophil-activating factors derived from monocytes have also been identified. Certain of these activities may be due to TNF (3, 52), whereas others may be novel polypeptides (53). Other potent activators of eosinophils include PAF (54) and C5a (55).

THE FUNCTIONS OF EOSINOPHILS

The most striking associations of eosinophilia have been with hypersensitivity diseases, especially bronchial asthma, and helminth infection. These are discussed in detail below. Considerable information also points to a role for the eosinophil in cutaneous diseases (56, 57), including the following: (a) syndromes associated with urticaria and edema [such as chronic, solar and delayed-pressure urticaria, the IgE-mediated late-phase reaction, episodic angioedema with eosinophilia, the IL-2 toxicity syndrome (40) and Wells' syndrome]; (b) chronic dermatitides (such as atopic dermatitis and onchocercal dermatitis); and finally (c) syndromes associated with fibrosis (such as eosinophilic fasciitis, the eosinophilamyalgia syndrome, and the Spanish toxic oil syndrome). Deposition of toxic granule proteins into tissues has been observed in all of these diseases (56, 57).

In addition, eosinophils could affect other cells. Because they express HLA-DR (25), eosinophils could interact with CD4⁺ lymphocytes and elicit antigen-specific responses. Furthermore, eosinophils express mRNA for cytokines such as transforming growth factors (TGF), TGF- α (58) and TGF- β (59), IL-1 (60), GM-CSF (61), and IL-5 (62), and they secrete IL-1 (60), GM-CSF (63), and IL-3 (63). Thus, eosinophils have the ability to produce cytokines that may affect many cells, as well as causing their own autoactivation.

Immunity to Parasites

Over the past two decades, new evidence has appeared supporting a role for the eosinophil as an effector cell in parasitism. This evidence consists of (a) the ability of eosinophils to kill helminths directly, (b) the ability of the eosinophil granule proteins to kill helminths, (c) the ability of anti-eosinophil serum to abolish immunity to parasites, and (d) the demonstration that eosinophils infiltrate about and degranulate onto degenerating parasites (3). Experiments using polyclonal anti-eosinophil serum were conducted in the 1970s. These experiments showed that anti-eosinophil serum reduced or totally abolished immunity to helminths, including *S. mansoni*, *Trichinella spiralis*, and *Trichostrongylus colubriformis* and to a tick, *Amblyomma americanus* (3).

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More recent experiments have utilized antibodies to IL-5 to abolish eosinophilia and to test the concept that the eosinophil is a potent mediator of immunity to helminths. In initial experiments, monoclonal antibody to IL-5 suppressed blood eosinophilia and the infiltration of eosinophils into the lungs of mice parasitized with *Nippostrongylus brasiliensis* (37). Subsequent experiments testing the effect of anti-IL-5 on parasite immunity showed that administration of anti-IL-5 completely ablated circulating and tissue eosinophils, but had no effect on the immunity of mice to *S. mansoni* (38, 64). Similar results were seen following administration of antibodies to IL-4, which markedly reduced serum IgE levels, but failed to diminish immunity. In contrast to these findings, treatment with antibodies to IFN- γ caused partial depletion of immunity in the vaccinated mice and increased the inflammatory reaction against the schistosomula in the lungs.

These results support a role for IFN- γ -dependent cell-mediated effectors in resistance to *S. mansoni*, and, at face value, the data appear sharply at variance with results of studies in the 1970s employing polyclonal anti-eosinophil serum. However, the possibility exists that mice employ different immune mechanisms for resistance to various parasites, including nematodes. Analyses of T helper cells indicate that murine T helper cells can be divided into two subsets in which IFN- γ is secreted by TH-1 cells, whereas IL-5 and IL-4 are secreted by TH-2 cells (65). TH-1 cells appear to be critically important for immunity to *Leishmania major* (66), and TH-2 type responses in mice lead to progressive disease and death following *L. major* infection.

Because eosinophils have long been associated with helminth infection in humans and because analyses of immune responses in humans indicate a bias toward IL-5 production (39, 67), it is tempting to speculate that TH-2 responses with IL-5 production in eosinophilia would be associated with immunity. Support for this point of view has recently come from studies of cellular immune responses to the murine nematode parasite, *Trichuris muris* (68). In this model of parasitism, immunity was associated with polarization of the T helper cell response during infection to give predominantly IFN- γ -secreting TH-1 cells in strains of mice unable to expel the parasite, and TH-2 cells that produced mainly IL-4 and IL-5 in resistant strains. Thus, these results would support the associations that existed in the past regarding the likely importance of eosinophils in helminth infections.

Bronchial Asthma

Peripheral blood eosinophilia was initially associated with asthma in 1889, and by 1922 the striking eosinophilia of the airways in patients with

asthma was already described. In 1975 peripheral blood eosinophilia was correlated with the severity of asthma as judged by the diminution of the FEV₁. With the recognition that MBP is a potent toxin to mammalian cells (69, 70), its effect on respiratory epithelium was tested: MBP produced striking damage to respiratory cells (71, 72). The changes produced by MBP are similar to the pathologic changes in bronchial asthma (1, 73), and these findings stimulated tests of the hypothesis that MBP might be a mediator of damage to bronchial epithelium. The results of such tests are reviewed elsewhere (1, 73, 74). Sputum levels of MBP in patients with asthma are in the range of MBP concentrations required for toxicity in vitro; furthermore, treating patients with asthma improved airflow and lowered the concentration of sputum MBP (75). MBP could also be localized to sites of damage in respiratory epithelium (76, 77). A detailed analysis of the effect of MBP on cilia revealed that MBP reduced the number of beating cells. MBP also stopped the beating of isolated axonemes, and the MBP target appears to be the axonemal ATPase (78). Further, MBP produced an increase in short-circuit current and net chloride secretion when applied to the mucosal side of tracheal membranes in Ussing chambers, which suggests that MBP could contribute to abnormalities in airway secretions and ciliary function by stimulating airway epithelial chloride and water secretion (79).

The studies described above were consistent with the hypothesis that the eosinophil through its granule proteins, particularly MBP, altered bronchial epithelium. More recently, the hypothesis that the eosinophil might be related to bronchial hyperreactivity, one of the cardinal stigmata of asthma, has been tested in several ways.

1. First, peripheral blood eosinophilia is correlated with the degree of bronchial hyperreactivity (73).

2. Bronchoalveolar lavage (BAL) fluids from patients with asthma revealed an increase in the number of eosinophils and in the quantities of MBP compared to controls; higher amounts of MBP were also present in the BAL fluids of patients with bronchial hyperreactivity compared to patients without hyperreactivity (80). Further, the severity of bronchial hyperreactivity and the concentrations of MBP in the BAL fluids were positively correlated.

3. Analyses of the role of ICAM-1 in eosinophil migration and airway responsiveness of cynomolgus monkeys showed that repeated inhalation of antigen caused an intense eosinophil infiltration in the lung and was associated with a marked increase in airway responsiveness. Daily injections of a monoclonal antibody to ICAM-1 attenuated both the eosinophil infiltration and the increased airway responsiveness in these animals (81).

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Thus, inhibition of eosinophil infiltration blocks the antigen-driven increase in bronchial hyperreactivity.

4. In primates repeated antigen inhalation caused a prolonged inflammatory reaction in the airway characterized by a marked increase in airway eosinophils and an increase in airway responsiveness, as shown by a leftward shift in the methacholine dose-response curve (82). The number of BAL fluid eosinophils and the level of BAL fluid MBP were related to the sensitivity of the animals to methacholine.

5. Investigation of the potential involvement of MBP in bronchial hyperreactivity showed that instillation of MBP directly into the trachea of primates resulted in a dose-related increase in airway responsiveness to inhaled methacholine (29). Among the four principal eosinophil granule proteins, only MBP caused this effect. In addition, immediately after instillation into the monkey lungs, both MBP and EPO induced a transient bronchoconstriction. These data suggest that MBP and EPO cause bronchoconstriction and that MBP directly causes bronchial hyperreactivity.

6. Investigations of the mechanism of MBP have shown that the MBP application to respiratory epithelium altered the responsiveness of muscle to agonists, such as acetylcholine and histamine (83), and that topical application of MBP to respiratory epithelium (but not direct instillation into tracheal smooth muscle) augmented the contraction of underlying smooth muscle to acetylcholine (84, 85).

7. Finally, acidic polyamino acids, which are potent antagonists of MBP toxicity, also inhibited the increase in methacholine sensitivity caused by MBP administration into the lung.

Overall, the results listed above suggest that eosinophil granule proteins, and especially MBP, cause bronchial hyperreactivity by their action on respiratory epithelium.

More recent studies have focused on the mechanism by which eosinophils might infiltrate the respiratory tract. As noted above, monoclonal antibodies to ICAM-1 blocked eosinophil infiltration and decreased bronchial hyperreactivity in a primate model (81). Antigen-induced pulmonary late-phase inflammation in patients with allergic rhinitis receiving segmental bronchial challenge with allergen was associated with marked eosinophilia, as well as increased levels of all eosinophil granule proteins and IL-5 in the BAL fluid (86). Analyses of biopsies of bronchial mucosa from patients with asthma by in situ hybridization showed the presence of cells containing IL-5 mRNA in six of ten patients (87). The six IL-5 mRNA-positive patients tended to have more severe disease than the four IL-5 mRNA-negative patients and showed a significant increase in the degree of infiltration of the bronchial epithelium by eosinophils and acti-

vated T cells. Further, among the patients who showed positive IL-5 mRNA, the numbers of T cells, eosinophils, and IL-5 mRNA expression were positively correlated. The type of cell expressing IL-5 mRNA in the bronchial submucosa remains unknown. However, a recent report suggested that virtually all of the hybridizing cells in BAL fluids could be accounted for as CD2+ T cells (88). Finally, IL-5 protein has now been measured in BAL fluid from symptomatic patients with asthma (89).

The above series of investigations suggest that the eosinophil is the principal mediator of the pathology of bronchial asthma (Figure 1), and that eosinophilia is related to the production of IL-5 by T lymphocytes. The possibility that other inflammatory cells, including eosinophils themselves (62) and mast cells, might produce IL-5 remains to be shown.

ASTHMA: THE EOSINOPHIL HYPOTHESIS

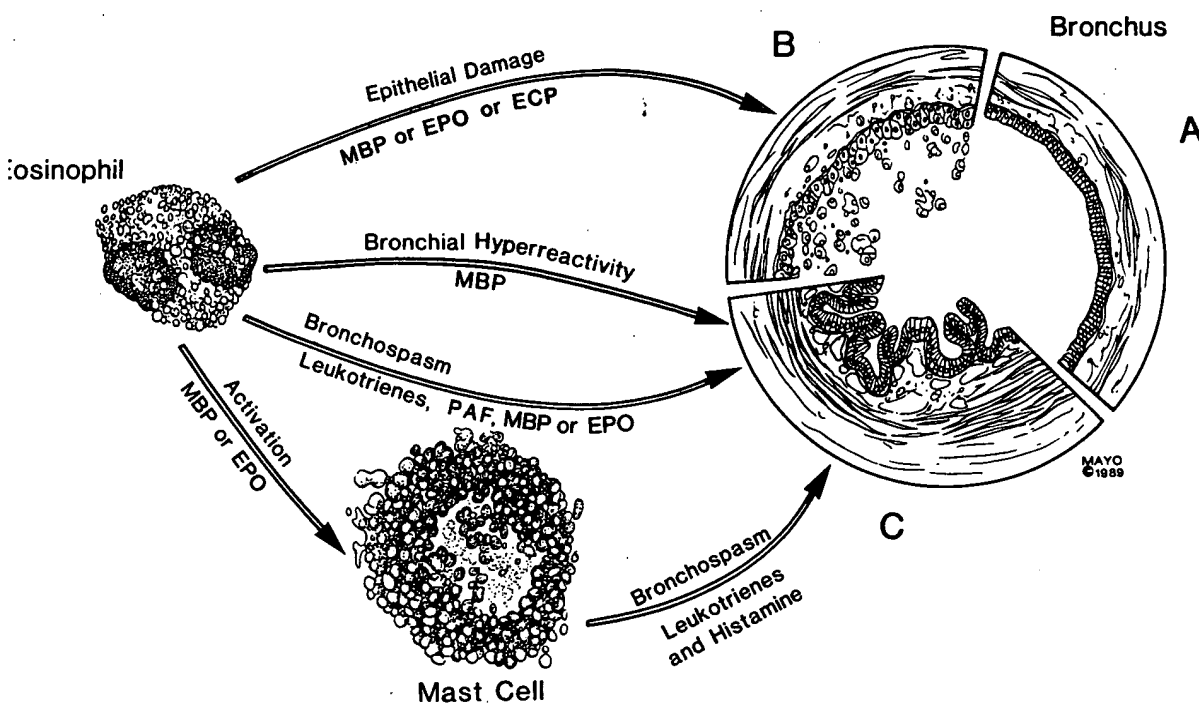


Figure 1 Schematic summary of the proposed role of eosinophils in bronchial asthma: the effects on the bronchus are shown by comparing a section of normal bronchus (A) with a portion damaged by MBP, ECP, or EPO (B), showing epithelial desquamation; and with a section (C) showing smooth-muscle hypertrophy, constriction, and edema of the lamina propria, resulting in a reduction in the caliber of the airway. (Modified from 90, by permission of Mayo Foundation).

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Further, the mechanism by which T cells mature onto a TH-2 pathway with production of IL-5 remains to be determined. As noted earlier, IL-5 appears to be an attractive target for pharmacological intervention, and it seems probable that specific antagonists of IL-5 will be available to clinical investigators in the not too distant future.

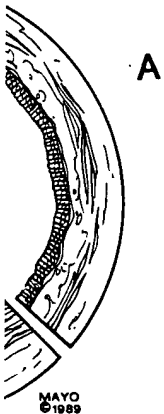
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Literature Cited

1. Frigas, E., Gleich, G. J. 1986. The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.* 77: 527-37
2. Belongia, E. A., Mayeno, A. N., Osterholm, M. T. 1992. The eosinophilia-myalgia syndrome and L-tryptophan. *Annu. Rev. Nutr.* 12: 235-56
3. Gleich, G. J., Adolphson, C. R., Leiferman, K. M. 1992. The eosinophil. In *Inflammation: Basic Principles and Clinical Correlates*, ed. J. I. Gallin, I. M. Goldstein, R. Snyderman. Boca Raton: Raven. 2nd ed. In press
4. Galli, S. J., Dvorak, A. M., Peters, S. P., et al. 1985. Lipid bodies: widely distributed cytoplasmic structures that represent preferential nonmembrane repositories of exogenous (^3H) arachidonic acid incorporated by mast cells, macrophages, and other cell types. In *Prostaglandins, Leukotrienes, and Lipoxins*, ed. J. M. Bailey, pp. 221-39. New York: Plenum
5. Hanker, J. S., Chandross, R. J., Solic, J. J., Weatherly, N. F., Laszlo, J., et al. 1981. Medusa cells: cytostructure and cytochemistry of amoeboid eosinophils with pseudopod-like processes. *Histochem. J.* 13: 905-19
6. Weller, P. F. 1991. The immunobiology of eosinophils. *N. Engl. J. Med.* 324: 1110-18
7. Hartnell, A., Moqbel, R., Walsh, G. M., Bradley, B., Kay, A. B. 1990. Fc gamma and CD11/CD18 receptor expression on normal density and low density human eosinophils. *Immunology* 69: 264-70
8. Hansel, T. T., Pound, J. D., Pilling, D., Kitas, D. G., Salmon, M., et al. 1989. Purification of human blood eosinophils by negative selection using immunomagnetic beads. *J. Immunol. Methods* 122: 97-104
9. Butterworth, A. E. 1984. Cell-mediated damage to helminths. *Adv. Parasitol.* 23: 143-235
10. Abu-Ghazaleh, R. I., Fujisawa, T., Mestecky, J., Kyle, R. A., Gleich, G. J. 1989. IgA-induced eosinophil degranulation. *J. Immunol.* 142: 2393-2400
11. Kita, H., Abu-Ghazaleh, R., Sanderson, C. J., Gleich, G. J. 1991. Effect of steroids on immunoglobulin-induced eosinophil degranulation. *J. Allergy Clin. Immunol.* 87: 70-77
12. Kita, H., Abu-Ghazaleh, R. I., Gleich, G. J., Abraham, R. T. 1991. Regulation of Ig-induced eosinophil degranulation by cyclic AMP. *J. Immunol.* 146: 2712-18
13. Kita, H., Abu-Ghazaleh, R. I., Gleich, G. J., Abraham, R. T. 1991. Role of pertussis toxin-sensitive G proteins in stimulus-dependent human eosinophil degranulation. *J. Immunol.* 147: 3466-73
14. Lopez, A. F., Eglinton, J. M., Gillis, D., Park, L. S., Clark, S., et al. 1989. Reciprocal inhibition of binding between interleukin 3 and granulocyte-macrophage colony-stimulating factor to human eosinophils. *Proc. Natl. Acad. Sci. USA* 86: 7022-26
15. Chihara, J., Plumas, J., Gruart, V., Tavernier, J., Prin, L., et al. 1990. Characterization of a receptor for interleukin 5 on human eosinophils: variable expression and induction by granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 172: 1347-52
16. Valerius, T., Repp, R., Kalden, J. R., Platzer, E. 1990. Effects of IFN on human eosinophils in comparison with other cytokines: a novel class of eosinophil activators with delayed onset of action. *J. Immunol.* 145: 2950-58

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17. Kroegel, C., Yukawa, T., Westwick, J., Barnes, P. J. 1989. Evidence for two platelet activating factor receptors on eosinophils: dissociation between PAF-induced intracellular calcium mobilization degranulation and superoxide anion generation in eosinophils. *Biochem. Biophys. Res. Commun.* 162: 511-21
18. Maghni, K., De Brum-Fernandes, A. J., Foldes-Filep, E., Gaudry, M., Borgeat, P., et al. 1991. Leukotriene B₄ receptors on guinea pig alveolar eosinophils. *J. Pharmacol. Exp. Ther.* 258: 784-89
19. Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature* 346: 425-34
20. Bochner, B. S., Luscinskas, F. W., Gimbrone, M. A. Jr., Newman, W., Sterbinsky, S. A., et al. 1991. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J. Exp. Med.* 173: 1553-56
21. Schleimer, R. P., Sterbinsky, S. A., Kaiser, J., Bickel, C. A., Klunk, D. A., et al. 1992. Interleukin-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J. Immunol.* 148: 1086-92
22. Lucey, D. R., Dorsky, D. I., Nicholson-Weller, A., Weller, P. F. 1989. Human eosinophils express CD4 protein and bind human immunodeficiency virus 1 gp120. *J. Exp. Med.* 169: 327-32
23. Cruikshank, W. W., Berman, J. S., Theodore, A. C., Bernardo, J., Center, D. M. 1987. Lymphokine activation of T4+ T lymphocytes and monocytes. *J. Immunol.* 138: 3817-23
24. Rand, T. H., Cruikshank, W. W., Center, D. M., Weller, P. F. 1991. CD4-mediated stimulation of human eosinophils. Lymphocyte chemoattractant factor and other CD4-binding ligands elicit eosinophil migration. *J. Exp. Med.* 173: 1521-28
25. Lucey, D. R., Nicholson-Weller, A., Weller, P. F. 1989. Mature human eosinophils have the capacity to express HLA-DR. *Proc. Natl. Acad. Sci. USA* 86: 1348-51
26. Gay, D., Maddon, P., Sekaly, R., Talle, M. A., Godfrey, M., et al. 1987. Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA-DR antigen. *Nature* 328: 626-29
27. Weller, P. F., Rand, T. H., Finberg, R. W. 1991. Human eosinophils function as HLA-DR dependent, MHC-restricted antigen-presenting cells. *FASEB J.* 5: A640 (Abstr.)
28. Butterfield, J. H., Weiler, D., Peterson, E. A., Gleich, G. J., Leiferman, K. M. 1990. Sequestration of eosinophil granule major basic protein in human mast cells. *Lab. Invest.* 62: 77-86
29. Gundel, R. H., Letts, L. G., Gleich, G. J. 1991. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J. Clin. Invest.* 87: 1470-73
30. Barker, R. L., Gleich, G. J., Checkel, J. L., Loegering, D. A., Pease, L. R., et al. 1991. Acidic polyamino acids inhibit human eosinophil granule major basic protein (MBP) toxicity: evidence of a functional role for proMBP. *J. Clin. Invest.* 88: 798-805
31. Gleich, G. J., Loegering, D. A., Bell, M. P., Checkel, J. L., Ackerman, S. J., et al. 1986. Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: homology with ribonuclease. *Proc. Natl. Acad. Sci. USA* 83: 3146-50
32. Sorrentino, S., Glitz, D. G., Hamann, K. J., Loegering, D. A., Checkel, J. L., et al. 1992. Eosinophil-derived neurotoxin in human liver ribonuclease: identity of structure and linkage of neurotoxicity to nuclease activity. *J. Biol. Chem.* 267: 14859-65
33. Ackerman, S. J., Zhou, Z., Clark, M. A., Irvin, C. G., Tenen, D. G. 1992. Human eosinophil lysophospholipase (Charcot-Leyden crystal protein): molecular cloning, expression, and potential functions in asthma. In *Eosinophils 1992*, ed. G. J. Gleich, A. B. Kay. New York: Marcel-Dekker. In press
34. Sur, S., Adolphson, C. R., Gleich, G. J. 1992. Eosinophils: biochemical and cellular aspects. In *Allergy: Principles and Practice*, ed. E. Middleton Jr., et al, Vol. 1, Chpt. 9. St. Louis: Mosby. 4th ed. In press
35. Clutterbuck, E. J., Hirst, E. M. A., Sanderson, C. J. 1989. Human interleukin-5, IL-5, regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 73: 1504-12
36. Saito, H., Hatake, K., Dvorak, A. M., Leiferman, K. M., Donnenberg, A. 1988. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc. Natl. Acad. Sci. USA* 85: 2288-92
37. Coffman, R. L., Seymour, B. W. P., Hudak, S., Jackson, J., Rennick, D.

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1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245: 308-10
38. Sher, A., Coffman, R. L., Hieny, S., Scott, P., Cheever, A. W. 1990. Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. *Proc. Natl. Acad. Sci. USA* 87: 61-65
39. Limaye, A. P., Abrams, J. S., Silver, J. E., Awadzi, K., Francis, H. F., et al. 1991. Interleukin-5 and the post-treatment eosinophilia in patients with onchocerciasis. *J. Clin. Invest.* 88: 1418-21
40. van Haelst-Pisani, C., Kovach, J. S., Kita, H., Leiferman, K. M., Gleich, G. J., et al. 1991. Administration of IL-2 results in increased plasma concentrations of IL-5 and eosinophilia in patients with cancer. *Blood* 78: 1538-44
41. Dent, L. A., Strath, M., Mellor, A. L., Sanderson, C. J. 1990. Eosinophilia in transgenic mice expressing interleukin-5. *J. Exp. Med.* 172: 1425-32
42. Tominaga, A., Takaki, S., Koyama, N., Katoh, S., Matsumoto, R., et al. 1991. Transgenic mice expressing a B-cell growth and differentiation factor gene, interleukin 5, develop eosinophilia and autoantibody production. *J. Exp. Med.* 173: 429-38
43. Hitoshi, Y., Yamaguchi, N., Korenaga, M., Mita, S., Tominaga, A., et al. 1991. In vivo administration of antibody to murine IL-5 receptor inhibits eosinophilia of IL-5 transgenic mice. *Int. Immunol.* 3: 135-40
44. Clutterbuck, E., Shields, J. G., Gordon, J., Smith, S. H., Boyd, A., et al. 1987. Recombinant human interleukin 5 is an eosinophil differentiation factor but has no activity in standard human B cell growth factor assays. *Eur. J. Immunol.* 17: 1743-50
45. Silberstein, D. S., Austen, K. F., Owen, W. F. 1989. Hemopoietins for eosinophils: glycoprotein hormones that regulate the development of inflammation in eosinophil-associated disease. *Hematol. Oncol. Clin. North Am.* 3: 511-33
46. Secor, W. E., Stewart, S. J., Colley, D. G. 1990. Eosinophils and immune mechanisms: VI. The synergistic combination of granulocyte-macrophage colony-stimulating factor and IL-5 accounts for eosinophil-stimulation promoter activity in *Schistosoma mansoni* infected mice. *J. Immunol.* 144: 1484-89
47. Fukuda, T., Dunnette, S. L., Reed, C. E., Ackerman, S. J., Peters, M. S., et al. 1985. Increased numbers of hypodense eosinophils in the blood of patients with bronchial asthma. *Am. Rev. Respir. Dis.* 132: 981-85
48. Peters, M. S., Gleich, G. J., Dunnette, S. L., Fukuda, T. 1988. Ultrastructural study of eosinophils from patients with the hypereosinophilic syndrome: a morphological basis of hypodense eosinophils. *Blood* 71: 780-85
49. Begley, C. G., Lopez, A. F., Nicola, N. A., Warren, D. J., Vadas, M. A., et al. 1986. Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils in vitro: a rapid and sensitive microassay for colony-stimulating factors. *Blood* 68: 162-66
50. Frick, W. E., Sedgwick, J. B., Busse, W. W. 1989. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am. Rev. Respir. Dis.* 139: 1401-6
51. Owen, W. F., Rothenberg, M. E., Petersen, J., Weller, P. F., Silberstein, D., et al. 1989. Interleukin 5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome. *J. Exp. Med.* 170: 343-48
52. Silberstein, D. S., David, J. R. 1986. Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. *Proc. Natl. Acad. Sci. USA* 83: 1055-59
53. Silberstein, D. S., Ali, M. H., Banker, S. L., David, J. R. 1989. Human eosinophil cytotoxicity-enhancing factor: purification, physical characteristics and partial amino acid sequence of an active polypeptide. *J. Immunol.* 143: 979-83
54. Kroegel, C., Yukawa, T., Dent, G., Venge, P., Chung, K. F., et al. 1989. Stimulation of degranulation from human eosinophils by platelet-activating factor. *J. Immunol.* 142: 3518-26
55. Kemen, P., Wymann, M. P., Von Tscharner, V., Deranleau, D. A., Tai, P.-C., et al. 1991. Shape changes, exocytosis, and cytosolic free calcium changes in stimulated human eosinophils. *J. Clin. Invest.* 87: 2012-17
56. Leiferman, K. M. 1991. A current perspective on the role of eosinophils in dermatologic diseases. *J. Am. Acad. Dermatol.* 24: 1101-12
57. Leiferman, K. M., Gleich, G. J. 1992. Cutaneous eosinophilic diseases. In *Dermatology in General Medicine*, ed. T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg, K. F. Austen. New York: McGraw-Hill. In press
58. Wong, D. T. W., Weller, P. F., Galli, S. J., Elovic, A., Rand, T. H., et al. 1990. Human eosinophils express transform-

- ing growth factor α . *J. Exp. Med.* 172: 673-81
59. Wong, D. T. W., Elovic, A., Matossian, K., Nagura, N., McBride, J., et al. 1991. Eosinophils from patients with blood eosinophilia express transforming growth factor β_1 . *Blood* 78: 1-4
 60. Del Pozo, V., De Andres, B., Martin, E., Maruri, N., Zubeldia, J. M., et al. 1990. Murine eosinophils and interleukin-1: alpha IL-1 messenger RNA detection by in situ hybridization. Production and release of IL-1 from peritoneal eosinophils. *J. Immunol.* 144: 3117-22
 61. Moqbel, R., Hamid, Q., Ying, S., Barkans, J., Hartnell, A., et al. 1991. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J. Exp. Med.* 174: 749-52
 62. Desreumaux, P., Janin, A., Colombel, J. F., Prin, L., Plumas, J., et al. 1992. Interleukin-5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J. Exp. Med.* 175: 293-96
 63. Kita, H., Ohnishi, T., Okubo, Y., Weiler, D., Abrams, J. S., et al. 1991. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J. Exp. Med.* 174: 745-48
 64. Sher, A., Coffman, R. L., Hieny, S., Cheever, A. W. 1990. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J. Immunol.* 145: 3911-16
 65. Mosmann, T. R., Coffman, R. L. 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7: 145-73
 66. Müller, I., Pedrazzini, T., Farrell, J. P., Louis, J. 1989. T-cell responses and immunity to experimental infection with *Leishmania major*. *Annu. Rev. Immunol.* 7: 561-78
 67. Limaye, A. P., Abrams, J. S., Silver, J. E., Ottesen, E. A., Nutman, T. B. 1990. Regulation of parasite-induced eosinophilia: selectively increased interleukin 5 production in helminth-infected patients. *J. Exp. Med.* 172: 399-402
 68. Else, K. J., Hülnter, L., Grecis, R. K. 1992. Cellular immune responses to the murine nematode parasite *Trichuris muris*. II. Differential induction of TH-cell subsets in resistant versus susceptible mice. *Immunology* 75: 232-37
 69. Butterworth, A. E., Wassom, D. L., Gleich, G. J., Loegering, D. A., David, J. R. 1979. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* 122: 221-29
 70. Gleich, G. J., Frigas, E., Loegering, D. A., Wassom, D. L., Steinmuller, D. 1979. Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* 123: 2925-27
 71. Frigas, E., Loegering, D. A., Gleich, G. J. 1980. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab. Invest.* 42: 35-43
 72. Motojima, S., Frigas, E., Loegering, D. A., Gleich, G. J. 1989. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am. Rev. Respir. Dis.* 139: 801-5
 73. Gleich, G. J. 1990. The eosinophil and bronchial asthma: current understanding. Review article. *J. Allergy Clin. Immunol.* 85: 422-36
 74. Hamann, K. J., Gleich, G. J., Gundel, R. H., White, S. R. 1991. Interactions between respiratory epithelium and eosinophil granule proteins in asthma: the eosinophil hypothesis. In *The Airway Epithelium: Physiology, Pathophysiology, and Pharmacology*, ed. S. G. Farmer, D. W. P. Hay, Chpt. 9, pp. 255-300. New York: Marcel Dekker
 75. Frigas, E., Loegering, D. A., Solley, G. O., Farrow, G. M., Gleich, G. J. 1981. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin. Proc.* 56: 345-53
 76. Filley, W. V., Holley, K. E., Kephart, G. M., Gleich, G. J. 1982. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 2: 11-16
 77. Harlin, S. L., Ansel, D. G., Lane, S. R., Myers, J., Kephart, G. M., et al. 1988. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J. Allergy Clin. Immunol.* 81: 867-75
 78. Hastie, A. T., Loegering, D. A., Gleich, G. J., Kueppers, F. 1987. The effect of purified human eosinophil major basic protein on mammalian ciliary activity. *Am. Rev. Respir. Dis.* 135: 848-53
 79. Jacoby, D. B., Ueki, I. F., Widdicombe, J. H., Loegering, D. A., Gleich, G. J., et al. 1988. Effect of human eosinophil major basic protein on ion transport in dog tracheal epithelium. *Am. Rev. Respir. Dis.* 137: 13-16
 80. Wardlaw, A. J., Dunnette, S., Gleich,

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81. Wegner, C. D., Gundel, R. H., Reilly, P., Haynes, N., Letts, L. G., et al. 1990. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 247: 456-59
82. Gundel, R. H., Gerritsen, M. E., Gleich, G. J., Wegner, C. D. 1990. Repeated antigen inhalation results in a prolonged airway eosinophilia and airway hyper-responsiveness in primates. *J. Appl. Physiol.* 68: 779-86
83. Flavahan, N. A., Slifman, N. R., Gleich, G. J., Vanhoutte, P. M. 1988. Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle. Role of the epithelium. *Am. Rev. Respir. Dis.* 138: 685-88
84. Brofman, J. D., White, S. R., Blake, J. S., Munoz, N. M., Gleich, G. J., et al. 1989. Epithelial augmentation of trachealis contraction caused by major basic protein of eosinophils. *J. Appl. Physiol.* 66: 1867-73
85. White, S. R., Ohno, S., Munoz, N. M., Gleich, G. J., Abrahams, C., et al. 1990. Epithelium-dependent contraction of airway smooth muscle caused by eosinophil MBP. *Am. J. Physiol.* 259(3): L294-L303
86. Sedgwick, J. B., Calhoun, W. J., Gleich, G. J., Kita, H., Abrams, J. S., et al. 1991. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. Characterization of eosinophil and mast cell mediators. *Am. Rev. Respir. Dis.* 144: 1274-81
87. Hamid, Q., Azzawi, M., Ying, S., Moqbel, R., Wardlaw, A. J., et al. 1991. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J. Clin. Invest.* 87: 1541-46
88. Robinson, D. S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., et al. 1992. Predominant TH₂-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326: 298-304
89. Ohnishi, T., Kita, H., Mayeno, A., Sur, S., Gleich, G. J., et al. 1992. Eosinophil-active cytokines and an inhibitor of cytokine activity in the bronchoalveolar lavage fluids (BALF) of symptomatic patients with asthma. *J. Allergy Clin. Immunol.* 89: 214 (Abstr.)
90. Gleich, G. J., Frigas, E., Filley, W. V., Loegering, D. A. 1984. Eosinophils and bronchial inflammation. In *Asthma: Physiology, Immunopharmacology, and Treatment*, ed. A. B. Kay, K. F. Austen, L. M. Lichtenstein, pp. 195-210. London: Academic

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